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SESQUITERPENES OF SIX *PORELLA* SPECIES (HEPATICAE)

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Key Word Index—*Porella vernicosa*; *P. gracillissima*; *P. fauriei*; *P. macroloba*; *P. densifolia*; *P. perrottetiana*; Hepaticae; sesquiterpenes; pungency; chemosystematics.

Abstract—The distribution in six *Porella* species of drimane-, aromadendrane- and pinguisane-type sesquiterpenes and norsesquiterpenes is described. The sharp pungent substance of *P. gracillissima*, *P. fauriei* and *P. macroloba* is (+)-tadeonal.

INTRODUCTION

Sesquiterpenes of six *Porella* species growing in Japan were investigated as part of our programme on biologically active substances of Hepaticae and as an extension of a chemosystematic investigation based on their sesquiterpenes. Fifteen *Porella* species are distributed in Japan, including the *Porella vernicosa* complex; *P. vernicosa*, *P. fauriei* and *P. macroloba* possess a characteristic pungent and skin irritant substance, while *P. densifolia* and *P. perrottetiana* are weakly bitter. Recently, we have reported the isolation of a sesquiterpene dialdehyde (**1**), responsible for the sharp pungency[1a,b], along with seven sesquiterpenes and norsequiterpenes from *P. vernicosa* [1c].

We now wish to describe the distribution of drimane, aromadendrane and pinguisane derivatives [2] of six *Porella* species and the comparison of these sesquiterpenes in fresh and old specimens of the *P. vernicosa* complex.

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RESULTS AND DISCUSSION

Table 1 shows the species, site and time of collection, and components detected in each species. The ether extract was directly analyzed by TLC and GLC to obtain a first orientation. All components isolated by combined chromatographic techniques (TLC, GLC and Column) were identified by direct comparison (mp, $[\alpha]_D$, IR, UV, MS and co-chromatography in TLC and GLC) with authentic specimens.

It was found that the sharp pungent substance of fresh *P. gracillissima*, *P. fauriei* and *P. macroloba* is the sesquiterpene dialdehyde (**1**), previously isolated from *P. vernicosa*. The chromatograms (TLC and GLC) of *P. gracillissima* extracts were very similar to those of *P. vernicosa*; in fact almost all compounds found in *P. vernicosa* could be detected in *P. gracillissima*. (+)-Cyclocolorenone (**4**) is the major sesquiterpene in *P. vernicosa* and *P. gracillissima*; on the other hand, *no* cyclocolorenone has been detected in *P. fauriei*, although it elaborates an unknown α,β-unsaturated sesquiterpene ketone, and cinnamolide (**3**), along with tadeonal (**1**). *P. macroloba* shows the strongest pungent taste of the taxa of the *P. vernicosa*

Table 1. Sesquiterpenes of *Porella*

Sample	Species	Collected, time and site		Weight (g)	Yield of extract (%)	Compounds detected							
						1	2*	3	4	5	6*	7	8*
A	*P. gracillissima*	Dec. 17 1974	Akiyoshi-cho, 160 m, Yamaguchi	2.0	1.2		+*	+	+	+*	+*	+*	+*
B		Aug 29 1954	Mesashiesamanbetsu, 500 m, Hokkaido	0.1	0.3		+*	+	+	+*	+*	+	+*
C		Jul. 24 1958	Mt. Senjo, 1200–1400 m, Nagano	1.5	0.5		+*	+	+	+*	+*	+	+*
D		Jul. 24 1958	Mt. Senjo, 1200–1400 m, Nagano	2.2	0.3		+*	+	+	+*	+*	+	+*
E		Aug. 15 1967	Mt. Kitadake, 1550 m, Yamanashi	0.6	0.6		+*	+	+	+*	+*	+*	+*
F	*P. vernicosa*	Mar. 30 1975	Sato-cho, 200 m, Hiroshima	5.8	1.6	+	+	+	+	+	+	+	+
G		Sep. 7 1959	Kinkazan, 300–450 m, Miyagi	1.2	0.3		+*	+	+	+*	+*	+*	+*
H	*P. fauriei*	Sep. 2 1974	Shiretokogoko, 250 m, Hokkaido	5.2	1.0	+		+					
I		Aug. 8 1954	Rijiri island, 500 m, Hokkaido	2.3	0.3			+					
J		Aug. 7 1959	Mt. Chokai, 800 m, Akita	0.6	0.4			+					
K	*P. perrottetiana*	Mar. 30 1975	Sato-cho, 200 m, Hiroshima	111.0	2.9					+	+	+	+
L	*P. densifolia*	Mar. 30 1975	Sato-cho, 200 m, Hiroshima	225.0	2.9					+	+	+	+
M	*P. macroloba*	Mar. 12 1976	Ato-cho, 510 m, Yamaguchi	4.5	1.0	+	+*	+		+	+*	+	+*
N		Mar. 11 1976	Mito-cho, 230 m, Yamaguchi	37.0	1.0	+	+*	+		+*	+*	+	+*

* Identified by cochromatography (TLC and GLC).

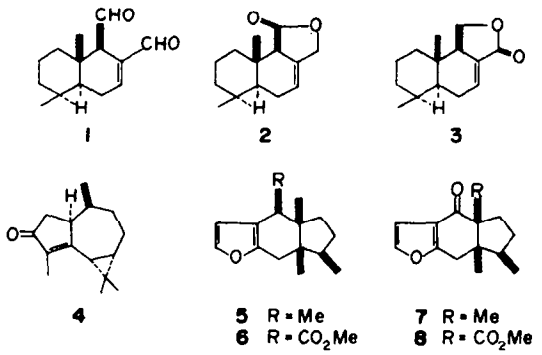

Fig. 1.

complex, and it contains the highest amount of (+)-tadeonal (*ca* 30% of the crude extract). The chromatogram of *P. macroloba* extract is similar to that of *P. gracillissima*. It elaborates the same drimanes and pinguisanes found in *P. gracillissima* and *P. vernicosa*; however, *no* cyclocolorenone has been found. Tadeonal has not been detected in old specimens of the *P. vernicosa* complex, because of its easy decomposition by exposure to air on storage. The chromatograms of the ether extract of the old specimens of *P. vernicosa* were surprisingly similar to those of the fresh samples. This was found also to be the case in fresh and old specimens of *P. gracillissima* and *P. fauriei*. Thus, drimane (except tadeonal (**1**)), aromadendrane and pinguisane derivatives can remain unchanged for more than twenty years.

From the sesquiterpene constitution, it was confirmed that *P. vernicosa* and *P. gracillissima* are very closely related taxa, and *P. fauriei* (growing at higher altitudes in northern Japan) is a taxon somewhat different from the above two species, although it is morphologically quite similar to *P. vernicosa*, but not to *P. gracillissima*. *P. macroloba* is also a taxon closely related to *P. gracillissima*, although cyclocolorenone has not been found.

No drimane and aromadendrane derivatives have been detected in *P. densifolia*; however, it is interesting from a biogenetical view-point that it elaborates a variety of pinguisane derivatives (**5**–**8**), although it is morphologically very different from the *P. vernicosa* complex. From *P. perrottetiana*, we could not find any of the components shown in Fig. 1; however, this species produces at least two α,β-unsaturated aldehydes, which lack pungency.

More recently, Huneck *et al.* [3] have reported the isolation of two pungent components, one of which has been determined as (+)-dihydrocinnamolide (=dihydroderivative of (**3**) from *P. arbor-vitae* (=*P. levigata*). The distribution of drimane derivatives is restricted to some higher plants; *Iresine celosioides* [4], *Drimys winteri* and *D. confertifolia* [5], *Polygonum hydropiper* [6] and *Cinnamosma fragrans* [7]. The presence of the drimane-type sesquiterpene, drimenol, has been reported by Huneck in the liverwort *Bazzania trilobata* [8]. From the present investigation, it is clear that *Porella* species are a rich source of drimane-, aromadendrane- and pinguisane-type sesquiterpenes.

The liverworts very often elaborate the optical isomers of components found in the higher plants [9]. It is interesting to note that the drimanes found in the present species and European ones [3,8] have the usual $9\beta,10\beta$-configuration; on the other hand, cyclocolorenone (**4**) is the enantiomer of that found in the higher plants [10,11].

EXPERIMENTAL

Extraction. Fresh liverworts (samples A, F, H, K, L, M and N) were air-dried for 2 weeks, and sorted. They, and the old specimens (samples No. B, C, D, E, G, I and J), were milled and each was finely powdered and stored over Si gel in a desiccator until constant weight was obtained. Each material was extracted with Et_2O for 2 weeks. Crude extracts were filtered through a short Si gel (30–70 mesh) column, and filtrates immediately used for the chromatographic analyses.

Isolation of sesquiterpenes. Crude extract of *P. gracillissima* (sample A) was chromatographed on Si gel (5 g) using a *n*-hexane and EtOAc gradient. The first compound to emerge was deoxopinguisone (**5**), characterized by its IR spectrum. The second fraction afforded the furanosesquiterpene (**7**) (UV and IR spectra identical with those of the authentic sample). Then was eluted a mixture of cyclocolorenone (**4**) and cinnamolide (**3**). This oil was directly separated by preparative TLC to give each pure component (IR, UV and $[\alpha]_D$ completely identical with those of the authentic sample). From the more polar fraction (*n*-hexane–EtOAc) colorless crystals (mp 57–58°) were obtained, the IR and MS spectra and $[\alpha]_D$ of which were in accordance with those of (+)-tadeonal (**1**). The green oil of *P. fauriei* (sample H) was treated as the same manner indicated above. (+)-Tadeonal (**1**) and cinnamolide (**3**) were isolated pure. Crude extract of *P. macroloba* (sample M) having a strong pungent taste, showed two pink and two deep orange spots on TLC, after spraying with Ehrlich reagent, indicating the presence of furanosesquiterpenes. It was chromatographed on Si gel (50 g) using the same solvent system described above, and purified by preparative TLC to give (**1**), (**3**), (**5**), (**6**) and (**7**) pure. Crude viscous oil of *P. densifolia* (sample L) showed 4 spots corresponding to furanosesquiterpenes on TLC. Each component was separated by Si gel (100 g) column chromatography and purified by preparative GLC and preparative TLC using *n*-hexane–benzene–EtOAc (5:14:6). Deoxopinguisone (**5**), (1.1% for the crude extract). $C_{15}H_{22}O$ (M^+ 218); Norsesquiterpene (**7**), mp 126–127°C, (4.4%). $C_{14}H_{18}O_2$ (M^+ 218). Methyl ester (**6**) of **5** (trace). $C_{16}H_{22}O_3$ (M^+ 262). Methyl ester (**8**) of **7** (1.2%). $C_{15}H_{18}O_4$ (M^+ 262). Components from the crude extracts of the other specimens were identified by the same manner indicated above. GLC: Analytical GLC. (a) column, Apiezon L. 40 m × 0.25 mm. Temp. programm. 100–230°/4°/min. Carrier gas, N_2 30 ml/min. (b) DEGS 5%, 2.25 m × 2 mm, Temp. programm. 100–230°/4°/min. N_2 30 ml/min. (c) SE-30 5%, operation conditions were the same as in b. Preparative GLC column, DEGS 20%, 2 m × 2 mm. Temp. 200°, carrier gas. He 20 ml/min. Analytical and preparative TLC: Precoated Si gel (0.25 mesh) F_{254} plate. Solvent, *n*-hexane–benzene–EtOAc (5:14:6). Spots were detected by UV light (254 and 360 nm), by spraying with Ehrlich reagent, 50% H_2SO_4 or 2,4-DNP.

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